Aromatic Nitrogen Compounds in Fossil Fuels - A Potential Hazard?

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Introduction

To achieve energy independence in the United States, converting coal to oil or extracting oil from shale will be required. Before commercial scale fossil fuel conversion facilities become a reality, chemical and biological studies of currently available synfuel samples derived from coal or shale are urgently needed in order to determine what the potential health problems, such as from occupational exposure, might be.

The nitrogen content either of shale oil (1-2%) (1) or coal derived oil (1-1.5%) (2) is far higher than that in petroleum (average N-content in petroleum is 0.05-0.1%) (3). This means enormous amounts of nitrogen containing species will be produced and found in crude synfuels, and this could lead to significant health or environmental impact. Clearly, a thorough characterization of nitrogen compounds in synfuels is an important pursuit.

Aromatic nitrogen compounds such as basic aza-arenes, neutral aza-arenes, and aromatic amines are considered environmentally important and several members of these classes of compounds possess biological activity. For example, dibenz(a,h)acridine, 7 H-dibenzo(c,g)carbazole, and 2-naphthylamine (4), are well known as carcinogens. In this paper, the methods used to isolate the basic aromatic nitrogen compounds and neutral aza-arenes from one shale oil and one coal-derived oil will be discussed. The mutagenic activities of these fractions, based on the Ames Salmonella typhimurium test, will be compared.

Experimental

Samples

A shale oil was obtained from the Laramie Energy Research Center's 150-ton retort operated for above ground simulation of in-situ retorting. The raw oil from the retort was an intimate emulsion of solids, water and oil; the emulsion was collapsed by centrifugation at 2500 RPM for about 20 min. at room temperature. Three phases were produced: an oily top phase (~50% vol.), a gelatinous intermediate phase (~20% vol.), and an aqueous phase (~30% vol.). The oil phase has been studied in this laboratory (5,6) and similar samples from other retort runs have been examined by others, principally by workers at the Laramie Energy Research Center (1,7,8).

A crude coal liquid (not necessarily representative of any final production scale product) was obtained from the Pittsburgh Energy Research Center. This material was very viscous, contained no water, and had a small amount of filterable solids (>5 μm range).

Separation Procedures

Neutral aza-arenes. Figure 1 shows the separation scheme for isolation of neutral aza-arenes from synfuels. After removing acidic and basic components from the whole sample, the neutral fraction was loaded onto a Sephadex LH-20 gel column. The column was eluted sequentially with 250 ml of isopropanol (Fraction AP) and 600 ml of acetone (Fraction AROM). Pentadecane and naphthalene were used to indicate the appropriate cut between elution of aliphatic compounds and 2-ring aromatic compounds.

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Acetone was removed from Fraction AROM and the residue was separated into three subfractions on a silicic acid column: Fraction I (PAH), eluate from 1200 ml benzene/hexane (1/3); Fraction II (neutral aza-arenes), eluate from 600 ml benzene/hexane (2/1); and Fraction III (polar), eluate from 600 ml ethanol. A mixture of a number of PAH compounds (pyrene, C^{14}-carbazole and 7 H-dibenzo(c,g)carbazole) were chromatographed in establishing this procedure.

Basic components. Basic fractions of the oils were produced by first dissolving the oils in diethyl ether and extracting the acids with a 1 M NaOH solution. A second extraction with 1 M HCl removed the basic components which were further partitioned between an aqueous/ether phase at pH 11. The basic fractions are contained in this ether phase. Further details of this procedure are reported elsewhere (9). Figure 2 shows the subfractionation scheme used to further separate basic fraction constituents. The basic fraction was placed onto a basic alumina column. The column was eluted with 500 ml benzene (benzene subfraction) followed by 700 ml ethanol. Ethanol was removed and the residue was separated further on a Sephadex LH-20 gel column. The column was eluted sequentially with 250 ml of isopropanol (isopropanol subfraction) and 600 ml of acetone (acetone subfraction).

Column packings and reagents. Basic alumina (100-200 mesh, AG-10, Bio-Rad Laboratories), Sephadex LH-20 gel (25-100 μ, Pharmacia Fine Chemicals) and silicic acid (100 mesh, Mallickrott, washed successively with hexane, acetone, and methanol; activated in 150°C oven for 16 hours) were used for column packings. Forty grams of alumina were added to 75 ml of benzene in a modified 50 ml buret column. A Sephadex column was prepared by swelling 75 g of the gel in isopropanol with sufficient excess to form a pourable slurry. The slurry was poured into the 250 ml buret column and allowed to compact by the gravity elution of 50-100 ml of isopropanol. A silicic acid column was made by pouring a slurry of 100 g of silicic acid in hexane into a 2.5 cm (O.D.)x 50 cm glass column.

All solvents were reagent grade and were freshly distilled except for the absolute ethanol. Reagents prepared for the microbial mutagenesis bioassay are described elsewhere (10,11).

GC/MS

GC/MS data were obtained using a Perkin-Elmer Model 3920 gas chromatograph interfaced to a DuPont 21-490B mass spectrometer via a glass jet separator. An effluent splitter provided about a 2:1 split between the mass spectrometer and a flame ionization detector, respectively. A Hewlett-Packard 21-094B data system interfaced to the mass spectrometer provided for the generation of mass spectra, mass chromatograms, library searches, etc. A glass GC column of 20 ft. x 1/8-in. O.D. was packed with 3% Dexsil 400 on 100/120 mesh Chromosorb 750 and installed with graphite ferrules. GC temperature programming was from 100°C (8 minutes hold) to 320°C at a linear rate of increase of 1°/min. Injector and detector temperatures were set at 320°C, helium gas inlet pressure at 100 psig, MS ionization voltage at 70 eV, mass scan rate at 2 seconds/decade and the MS resolution at about 600.

Microbial Mutagenesis Assay. Salmonella typhimurium bacteria, strain TA98, were generally employed. The experimental procedures are described by Ames et al. (10). Briefly, the bacteria are added to a soft agar containing nutrients and in some cases, enzyme activation preparations along with the substance being tested. The essential condition is that the amino acid, histidine, is absent. The suspension, containing approximately 2 x 10^8 bacteria, is overlaid on minimal agar plates and incubated. If the test substance is a mutagenic agent in this system, then large colonies (which have reverted to the wild type) are evident on the plate and can be counted, i.e., by mutation they can produce their own histidine and grow in a

histidine-free medium. If no colonies form except for a control background level, then no mutations have occurred. A test consists of assays at several concentrations of test substance in order to obtain a dose-response curve. Some potential mutagens require metabollic activation with liver homogenate preparations.

Results and Discussion

Neutral Aza-arenes

The procedures used for the isolation of neutral aza-arenes from synthetic crude oils evolved in part from an extraction scheme and a gel filtration chromatographic scheme (5, 12) developed in this laboratory. The silicic acid adsorption chromatography step was developed by Snook et al. (13) to isolate indole/carbazole from cigarette smoke condensate. But the silicic acid chromatography has been evaluated and some modifications have been made to make the system compatible with a gravity flow column. A good separation between PAHs and neutral aza-arenes was achieved by eluting the column with 1/3 (benzene/hexane) followed by 2/1 (benzene hexane). However when large quantities of aliphatic compounds are present in the sample, these contaminate all the eluate fractions. It is necessary to remove major portions of aliphatic components prior to the silicic acid step. Gel filtration chromatography with a Sephadex LH-20 column eluted with isopropanol is effective for removing a major portion of aliphatic and polymeric compounds while retaining aromatic compounds of two rings and higher. Further elution of the column with acetone results in the quantitative recovery of aromatic components in order of increasing aromaticity (5). Silicic acid chromatography produced a relatively pure neutral aza-arene fraction (Fraction II), suitable for GC/MS analysis and bioassay. The separation of PAH and neutral aza-arene fractions was further confirmed by a chromatographic study of oil samples spiked with large excesses of benzo(a)-pyrene along with carbazole. Tracer studies of oil samples spiked with C^{14}-carbazole showed that carbazole was not eluted from the column with even as much as 1600 ml 1/3 (benzene/hexane). The recovery of C^{14}-carbazole from the silicic acid column with 2/1 (benzene/hexane) was 97% for the coal derived oil and 75% for the shale oil. This agrees well with data on cigarette smoke condensate (13).

The presence of indole/carbazole analogues in Fraction II from both oil samples is also supported by their IR spectra. These fractions have a sharp band at 3430 cm^{-1} which is normally found in the IR spectrum of carbazole and is characteristic of the N-H group in these compounds.

In a peak by peak comparison of GC profiles of Fraction II obtained with an FID and an NPD, we found the majority of the GC peaks were nitrogen containing compounds.

The proton NMR spectrum of Fraction II gave a ratio of aliphatic protons to aromatic protons close to unity (1.06) indicating that only a few alkyl groups are contained in Fraction II. This result seems to be consistent with the observation from GC/MS analysis in which the biggest alkyl substituent was six carbons.

The odd m/e values of molecular ions obtained from GC/MS data confirmed the predominant presence of nitrogen compounds in Fraction II. Tentatively identified components are C_1-C_3 phenylpyrroles, indole, C_1-C_6 indoles, C_1-C_3 phenylindoles, carbazole, C_1-C_5 carbazoles, benzocarbazoles, and C_1-C_3 benzocarbazoles.

The weight distribution of aliphatic and aromatic subfractions are listed in Table 1. N-heterocyclic material is much less than aromatic hydrocarbon material in the shale oil. The quantities of carbazole in the original oil samples were estimated by external standard calibration based on GC peak height. They

are 147 ppm for the shale oil and 268 ppm for the coal-derived oil. Because of lack of standard neutral nitrogen-heterocyclic compounds, the quantitative data, except for carbazole, are not available at this time.

Table 2 summarizes the mutagenicity test data on the four neutral subfractions of coal-derived oil and several commercially available neutral aza-arenes. Indole and carbazole, exhibit no mutagenicity. Highly carcinogenic 7 H-dibenzo(c,g)-carbazole gave a slight but definite positive mutagenic activity at a low dose range (below 25 μg/plate). Specific activity could not be determined for this compound due to the toxic effect at a higher dose range. Benzocarbazoles and their alkyl-substituted compounds were not available for this study. Despite the incompleteness of this study, the correlation between chemical structure and mutagenic activity is expected to be in general agreement with that observed with PAH (14). Fraction AP which contains mostly aliphatic and polymeric constituents shows no mutagenic activity as expected. PAH subfractions exhibit the lowest specific activity among the three subfractions. The neutral aza-arene fractions, which are normally isolated with PAH fractions, have more than two times the specific activity of the PAH fraction. This indicates that the analysis of neutral aza-arene fractions of synfuels are as important as PAH analysis. The higher specific activity of the neutral aza-arene fraction may contain substantial quantities of multi-ring compounds. The further subfractionation of the neutral aza-arene fractions into fractions with aza-arenes of approximately the same ring sizes is presently being undertaken.

Basic nitrogen compounds. To further understand what classes or types of basic nitrogen compounds are the most bioactive among compounds in the basic fraction, we recently developed a separation method which isolates the mutagenically active compounds from the bulk of the base fraction (15). The subfractionation scheme is shown in Figure 2. This separation method was devised using a microbial mutagenesis bioassay as a liquid chromatographic detector in the development of the chromatographic subfractionation procedure. Table 3 summarizes the mutagenicity test data on the three basic subfractions of the synfuels. The bioactivities of several commercially available compounds and three that were synthesized in this laboratory are listed in Table 4 for comparison. About 90% of the basic mutagenic activity is recovered in the acetone subfraction which comprises about 10% of the basic fraction. That suggests that the method can be used for isolation of basic mutagenic components from samples of different origins (in this case oil shale or coal). To demonstrate the utility of the method, 3 different kinds of cigarette smoke condensates were fractionated using this scheme and the mutagenic compounds were also concentrated in the acetone subfraction. GC/MS data indicate that the major compound type of the benzene subfractions is C_3-C_{13} alkyl substituted pyridines. Mutagenic activities of some commercially available pure pyridines (such as pyridine and C_1-C_3 pyridines) and one specially synthesized C_9-pyridine are essentially zero in agreement with these results. The major compounds in the isopropanol fractions are partially hydrogenated 1-2 ring aza-arenes. Biological data on compounds of this type are not available for comparison.

The acetone subfraction of shale oil was about half as mutagenic as benzo(a)-pyrene while the coal-derived oil subfraction was about four times more active. GC/MS data indicate multi-ring aza-arenes comprised a large portion of the acetone subfraction, e.g. aza-benzoperylene, aza-indenopyrene, and aza-coronene have been identified. This finding is consistent with bioactivity data from a few multi-ring aza-arene compounds such as dibenz(a,j)acridine (13,000 rev/mg), 9-methyl-10-aza-benzo(a)-pyrene (30,000 rev/mg) and 10-azabenzo(a)pyrene (130,000 rev/mg). The purposes in synthesizing two nitrogen isologs of benzo(a)pyrene was, first, to confirm the higher mutagenic activity of multi-ring aza-arenes, which comprise a large portion of the acetone subfraction, and to compare their activities with the mutagenic activity of benzo(a)pyrene. 10-azabenzo(a)pyrene is two times more active than benzo(a)pyrene. A methyl group on the 9 position of azabenzo(a)pyrene, decreases the mutagenic activity. This might be explained by steric hindrance toward the forming of an epoxide at the 7 and 8 position.

The presence of aromatic amines in the active subfraction from both oil samples was first suggested from their IR spectra. Bands at 3220 cm^{-1} and 3370 cm^{-1}, which are normally found in the IR spectra of aromatic amines, are characteristic of the amino compounds in the fraction. By acetylation of the acetone subfraction, we isolated large amounts of primary aromatic amides from the acetone subfraction. A mixture of ten compounds consisting of 4 aza-arenes and 6 aromatic amines (2,4,6-trimethylpyridine, quinoline, acridine, dibenz(a,h)acridine, N, N-dimethylaniline, N-methylaniline, N-phenyl-2-naphthylamine, aniline, 2-naphthylamine, and 1-aminopyrene) was separated on a basic alumina column followed with a Sephadex LH-20 column. All primary amines (1,2, and 4-ring compounds), quinoline and dibenz(a,h)-acridine were concentrated in the acetone subfraction. This finding may mean that the primary aromatic amines as well as multi-ring aza-arenes are producing the mutagenic activities of the acetone subfractions. A logical extension of this work is to further separate the acetone subfraction into primary amines and multi-ring aza-arenes. This should lead to some important conclusions regarding the mutagenic effects of these classes of compounds.

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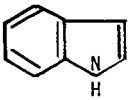
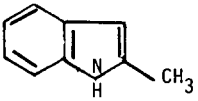
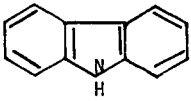
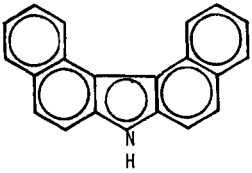
Table 1. Weight Distribution of Neutral Fractions*

	Weight % Distribution	
	Shale Oil	Coal-Derived Oil
Aliphatic and Polymeric (AP)	85.1	35.1
Aromatic (AROM)	14.9	62.9
PAH (I)	10.1	35.9
Neutral Aza-arene (II)	1.4	9.5
Polar (III)	4.3	17.7

*Acid-base extraction yield 89.6% by weight of neutral fraction from shale oil and 56% from coal-derived oil.

Table 2. Mutagenic Activities of the Neutral Subfractions of a Coal-Derived Oil and Some Neutral Aza-arene Compounds

Fraction/Compound	Specific Activity (rev/mg)*
Aliphatic and Polymeric Fraction (AP)	0
PAH Fraction (I)	1390
Neutral Aza-arene Fraction (II)	3250
Polar Fraction (III)	3380
	0
	0
	0
	0
	**

*Tested on TA98 with Aroclor S-9.

**Four-fold increase over spontaneous reversion at dose below 25 µg/plate. Toxic effect developed at higher dose.

Table 3. Distribution of Mutagenic Activity in Basic Subfractions

Subfraction	Shale Oil Base Fraction			Coal-Derived Oil Base Fraction		
	Average[1] Weight (%)	Average Specific Activity (rev/mg)	Average[2] Relative Activity (%)	Average[1] Weight (%)	Average Specific Activity (rev/mg)	Average[2] Relative Activity (%)
Benzene	78	0	0	76	850	2
Isopropanol	13	277	1	12	0	0
Acetone	9	25,000	92	12	220,000	88
TOTAL	100		93	100		90

[1] Percentage by weight of the basic fraction.

[2] Percentage of mutagenic activity (TA98, S9, Aroclor 1254) of the basic fraction accounted for in the subfraction.

Table 4. Mutagenicity of Basic Aromatic Compounds*

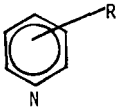
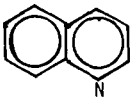
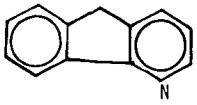
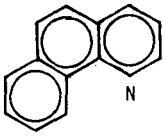
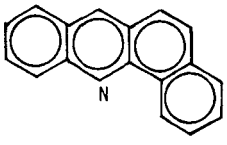
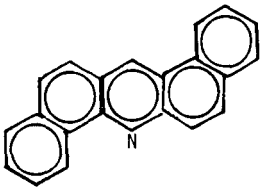
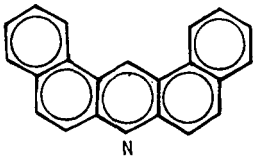
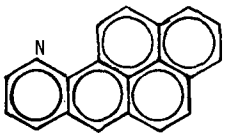
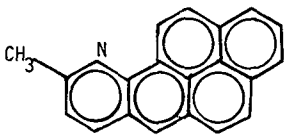
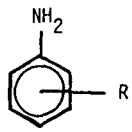
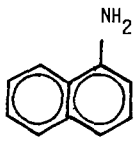
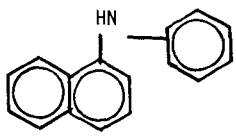
Compound Type - Basic Aza-arene	Specific Activity (rev/mg)
 R = H, CH_3, C_2H_5, C_3H_7, C_9H_{19}	0
	340
	0
	0
	0
	6,000
	13,000

Table 4. Continued

Compound Type - Basic Aza-arene (cont'd)		Specific Activity (rev/mg)
		130,000
		30,000
Aromatic Amine		
	R = H, CH_3, C_2H_5	0
		4,660
		0

*Aroclor induced.

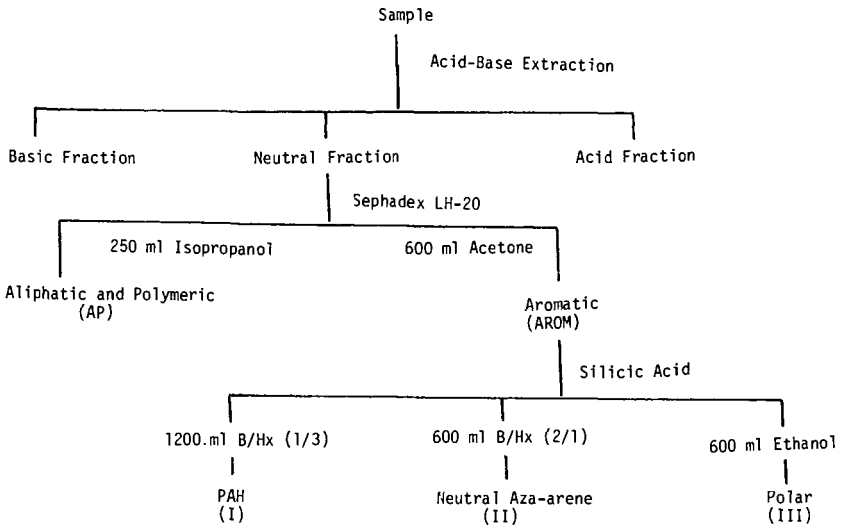


Figure 1. Isolation of Neutral Aza-arenes from Synfuels.

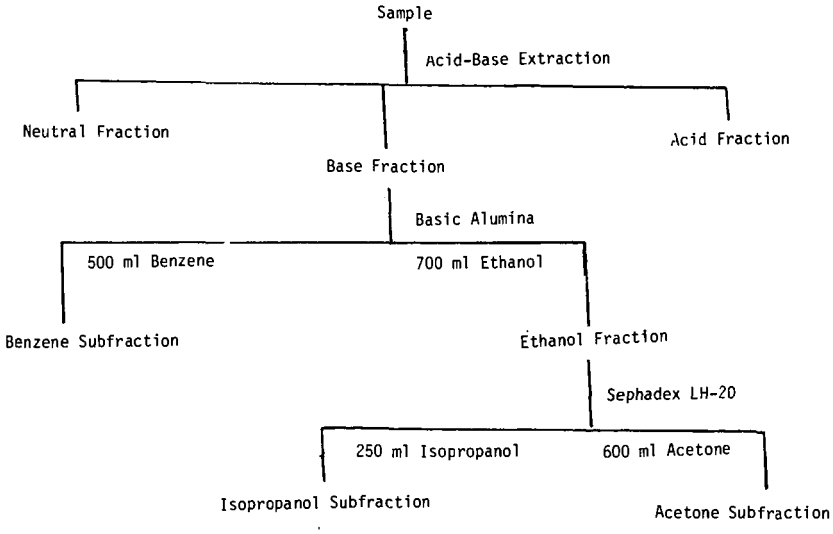


Figure 2. Subfractionation of the Basic Fraction of Synfuels.